PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patent Office
Canberra

I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002953073 for a patent by ACCESS PHARMACEUTICALS AUSTRALIA PTY LIMITED as filed on 21 November 2002.

WITNESS my hand this
Tenth day of December 2003

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES

Our Ref: 7756290

P/00/009
Regulation 3:2

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s): Access Pharmaceuticals Australia Pty Limited
28 Barcoo Street
Roseville New South Wales 2069
Australia

Address for Service: DAVIES COLLISON CAVE
Patent & Trade Mark Attorneys
Level 10, 10 Barrack Street
SYDNEY NSW 2000

Invention Title: **Amplification of biotin-mediated targeting**

The invention is described in the following statement:

AMPLIFICATION OF BIOTIN-MEDIATED TARGETING

Technical Field

The invention relates to the delivery of drug, peptide and protein pharmaceuticals using the biotin-mediated uptake system. More particularly the invention relates to the amplification of active substance delivery with the biotin uptake system using a biotin-drug complex. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same and methods of treatment involving the complexes.

Background Art

Conventional cancer chemotherapy involves the killing of cancerous cells and tumours with cytotoxic agents. In such treatments, it is often necessary to increase the quantity of cytotoxic drugs in an exponential fashion in order to obtain a linear increase in killing of cancer cells. This in turn leads to an undesirable increase in non-specific cytotoxicity of bistander, healthy cells. In order to reduce the effect of the high dose of toxin on normal, healthy tissues, it is often necessary to repeatedly deliver a smaller dose of cytotoxin, which often leads to the survival of a small fraction of drug-resistant cells.

Attempts have been made to increase the dose of cytotoxic agent delivered to the tumor cell through the use of specific targeting agents such as monoclonal antibodies specific for "tumor-antigens". In many cases it has been found that the resultant antibody-drug conjugate is highly immunogenic. This often leads to an antibody response against the conjugate, meaning that treatment with the complex must be halted. For this reason small, poorly immunogenic molecules which have a high specificity for tumour cells have been sought as alternatives to antibody-drug conjugates.

Vitamins essential for the growth of rapidly dividing cells such as tumours have recently been identified as candidate molecules for tumour targeting. Two such vitamins, folic acid and vitamin B12, have been shown to have some activity in targeting a small subset of aggressive tumour cell lines. Thus Russell-Jones and co-workers describe the use of vitamin B12 and folic acid as targeting molecules for the delivery of polymers and nanoparticles containing or linked to active agents both for oral delivery and also for cancer therapy (see for example WO00/66090, WO00/66091, WO94/27641).

Cancer and related diseases are a leading cause of death in today's society. It is estimated that 1,284,900 new cases of cancer will be diagnosed and 555,500 people will die from cancer in the United States in the year 2002. (Jemal, A., Thomas, A., Murray, T., Thun, M (2002) "Cancer statistics", *CA Cancer J Clin.*, 52(1), 23-47). Accordingly there is a strong need to identify new, improved, better and/or alternative pharmaceutical compositions and agents for its treatment, amelioration and prevention. There is a further need for chemotherapeutic agents which address some of the undesirable side effects of known agents. There is also a need for different therapies to be available to physicians to combat the numerous and various types of cancers and to provide new options for treatment to address issues of tolerance of proliferating cells to the existing chemotherapeutic agents and treatment regimes. Any beneficial effects which can be obtained with new or alternative active agents to reduce the administration amount and/or duration of treatment with chemotherapeutic drugs, or the provision of safer administration routes and hopefully fewer or less sever side effects is greatly sought after.

It is a preferred object of the present invention to provide pharmaceutical compositions and methods for the treatment, amelioration or prophylaxis of disease by the amplification of active substance delivery to biological targets. The present invention also seeks to provide pharmaceutical compositions and methods for targeting neoplastic cells for treatment, which compositions and methods provide improved cell activity in terms of targeting function and/or improved delivery of toxic agents.

Surprisingly, it has been found by the inventors that the vitamin biotin, and derivatives thereof, are able to target a much wider range of tumours than either vitamin B12 or folate. Biotin is one of the water-soluble B group vitamins and is used for fat, protein and carbohydrate metabolism, cell growth and fatty acid production. Biotin has been employed in the laboratory as a trace and in imaging studies with IGG monoclonal antibodies. However, useful drug delivery complexes involving biotin have not been realised before this present study. Therefore, complexes involving this vitamin, and derivatives thereof, is the subject of the current application.

It is unexpected that biotin would have such marked activity and wide application to biological targets including cancerous cells and tumours, sites of inflammation, and macrophages and dendritic cells.

There are two major limitations to the use of biotin to target to cells. Firstly, the dose deliverable is small as it is restricted to one molecule of drug for each molecule of biotin and is limited to the number of receptors on the cell. Secondly, due to the small molecular weight of the biotin-drug complexes they are rapidly excreted in the kidneys, thus leading to rapid removal of the complexes from the circulatory system.

Summary of the Invention

The invention relates to the surprising observation that biotin complexes are able to act as targeting agents for the delivery of macromolecules to many biological targets associated with disease including cancerous cells and tumours, sites of inflammation, and macrophages and dendritic cells. The biotin complexes of the invention are large molecular weight complexes incorporating biotin or analogues thereof and an active agent to be delivered. The biotin complexes of the invention most preferably involve polymer or nanoparticle technology suitable for the amplified delivery of the active agent. Simple conjugates of one targeting molecule with one drug molecule are less effective for the reasons described above. By incorporating many molecules of the drug with the macromolecular constructs of this invention, the biotin targeting effect is amplified; that is,

many more molecules of the drug can be delivered for each biotin-receptor interaction than is possible with simple conjugates involving one drug and one targeting molecule.

Amplification of the targeted drug delivery can occur by linkage of the pharmaceutical to a polymer backbone to which a number of biotin molecules are linked, either subsequently, previously or concurrently. The large size of the biotin-[drug-polymer] complex also minimises accumulation of the biotin-drug polymers in the kidneys.

The targeted drug delivery can also occur by incorporation of the drug to be delivered within a nanoparticle, which is coated with biotin or an analogue thereof. Again, accumulation of the biotin-drug complex in the kidneys is minimised due to the large size of the nanoparticle.

According to an aspect of the invention there is provided a macromolecular complex comprising biotin, or an analogue thereof, in association with an active agent and a support for the amplified delivery of the active agent. The complex of the invention preferably involves the use of polymers or nanoparticles as the support for the active agent and biotin targeting agent.

According to another aspect of the invention there is provided a pharmaceutical composition which comprises a complex of the invention in association with a pharmaceutically acceptable carrier or diluent.

According to another aspect of the invention there is provided a method for the treatment, prophylaxis or amelioration of disease, which comprises the step of administering to a subject a therapeutically effective amount of a complex or composition of the invention. In a preferred embodiment the disease is for example cancer, inflammation or a macrophage mediated event.

In a preferred embodiment of the invention the biotin-targeting moiety is in itself pharmaceutically active, such as by being cytotoxic or having anti-inflammatory activity.

The complexes of the invention can be used to stimulate macrophages and dendritic cells with antigens as the active agent through targeting of these complexes of biotin and antigen to biotin receptor positive cells. Moreover, the complexes of the invention can be used to target macrophages with cytotoxic agents to reduce the severity of macrophage-mediated events in diseases such as psoriasis, colitis, Crohn's disease, multiple sclerosis, graft-versus-host reaction and rheumatoid arthritis.

Thus, according to another aspect of the invention there is provided a method for stimulating macrophages or dendritic cells with an antigen by contacting the macrophage or dendritic cell with a complex of the invention, wherein the active agent is an antigen and the macrophage or cells to be contacted are biotin receptor positive.

In a further embodiment of the invention the complexes can be used to deliver anti-parasitic drugs to macrophages. Such processes can be used in the treatment of intracellular parasites such as malaria, salmonella, and leishmania.

In another embodiment of the invention, the complexes can be used to enhance the transfer of the drug from the intestinal lumen to the bloodstream.

According to another aspect of the invention there is provided the use of a complex of the invention in the manufacture of a medicament for the treatment of disease.

According to another aspect of the invention there is provided the use of biotin or an analogue thereof in the manufacture of a complex of the invention.

According to another aspect of the invention there is provided an agent for the treatment, prophylaxis or amelioration of a disease which agent comprises a complex of the invention.

These and other aspects of the invention will become evident from the description and claims which follow, together with the accompanying drawings.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Brief Description of the Figures

Figure 1 represents the level of uptake of Biotin-Rhodamine-HPMA polymer as determined by fluorescent microscopy by P815 tumour cells (1,000x).

Figure 2 represents the level of uptake of Biotin-Rhodamine-HPMA polymer as determined by fluorescent microscopy by P815 tumour cells (1,000x).

Figure 3 represents the level of uptake of Biotin-Rhodamine-HPMA polymer as determined by fluorescent microscopy by Colo-26 tumour cells (100x).

Figure 4 represents the level of uptake of Biotin-Rhodamine-HPMA polymer as determined by fluorescent microscopy by Colo-26 tumour cells (100x).

Figure 5 represents the level of uptake of Rhod-HPMA polymer into L1210FR tu IP by biotin targeting, compared to vitamin B12 targeting, folate targeting and a control.

Figure 6 represents the level of uptake of FITC-HPMA polymer into L1210FR tu IP by biotin targeting, compared to vitamin B12 targeting, folate targeting and a control.

Detailed Description of the Invention

The complexes of the present invention relate to a support to which an active agent and a biotin molecule, or analogue thereof, are associated or conjugated. These biotin complexes are directed to biological targets having biotin receptors, and are particularly suitable for parenteral delivery to tumours, cancerous cells, sites of inflammation, and to macrophages and dendritic cells. The complexes of the invention utilise the biotin receptor system for uptake binding and uptake, and have the added advantage of increasing the amount of active agent which can be delivered via the biotin uptake mechanism, as well as minimising or avoiding targeting to the kidneys by virtue of their size. The support is preferably a polymer or nanoparticle.

Polymers

The polymeric complexes of the invention have the general formula:

$$(\text{Biotin-Q})_n\text{-P-(Q'-A)}_m$$

wherein, biotin, or an analogue thereof, is a targeting molecule which will bind to surface biotin receptors on tumor cells, and where

n, the molar substitution ration of biotin in the complex, is in the range from 1.0 to 50.0;

P is a pharmaceutically acceptable polymer;

A is a pharmaceutically active substance;

m, the molar substitution ratio of A in the complex, is in the range from 1.0 to 1000; and

Q and Q' are independently a covalent bond, or a spacer compound linking biotin, P and A by covalent bonds, whereby the spacer may be either stable or degradable.

The invention also provides a complex which comprises more than one active substance linked to a polymer, which is linked to at least one targeting molecule which is a biotin molecule, or analogue thereof, wherein the ability of the targeting molecule to undergo the binding reactions necessary for uptake and transport of biotin in a vertebrate host and the activity of the active substance are substantially maintained, following conjugation or

following biological release of the active substance from the polymer. This process comprises one or more of the following steps:

a) reacting the active substance with the polymer to form said complex:

b) chemically modifying the active substance to provide at least one functional group capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex:

c) chemically modifying the targeting molecule (hereinafter referred to as TM) to provide at least one functional group capable of forming a chemical linkage and reacting the carrier and polymer to form said complex:

d) chemically modifying the active substance and the polymer to provide functional groups capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex:

e) reacting the active substance with at least one cross-linking agent and reacting the active substance of polymer to form said complex:

f) reacting the TM with at least one cross-linking agent and reacting the polymer and TM to form said complex:

g) reacting the active substance and polymer with at least one cross-linking agent and reacting the active substance and polymer to form said complex:

h) reacting the active substance directly with a polymeric support to form an intermediate containing one or more molecules of the active substance linked to the polymer, and subsequently coupling the polymer-active substance intermediate to one or more targeting molecules:

i) coupling one or more TM molecules to a polymeric support and subsequently reacting the carrier-polymer intermediate with one or more molecules of the active substance to give a final complex containing one or more molecules of the active substance.

In another aspect of the invention there is provided a process for the production of a complex having the general formula:

$$(\text{biotin-Q})_n\text{-P-(Q'-A)}_m$$

wherein biotin, Q, P, Q', A, n and m are as defined above, said process selected from:

a) reacting A with P to form an intermediate complex, and thereafter reacting the intermediate complex with biotin;

b) reacting biotin with P to form an intermediate complex and thereafter reacting the intermediate complex with A;

c) the process of step a) or step b) wherein one or more of biotin, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; or

d) reacting one or two of biotin, P or A with Q and/or Q' prior to coupling with the other reactants.

In a further aspect of the invention there is provided a method for the modification of a polymeric support to introduce functional groups capable of reacting either directly with the active substance or with a chemically-modified form of the active substance. The resulting polymer-active substance intermediate contains one or more molecules of the active substance, said intermediate being suitable for coupling to the TM to give a complex capable of amplified delivery of the active substance.

Other methods of formation of the compositions of this invention may be employed as known to those skilled in the art.

The polymer complexes of the present invention have been targeted to cancer cells using biotin or analogues thereof as the targeting moiety. Once the drug-polymer has reached its target tissue the complex is endocytosed by the target cell and the pendant drug may be released by the action of lysosomal enzymes, by cleavage of a disulfide linked drug by intracellular glutathione or otherwise. While it is possible that these complexes could be used for oral delivery of the drug to the circulatory or lymphatic drainage system in general, the complexes of this invention preferably relate to targeting drugs/pharmaceuticals to tumour/cancer cells.

In a further embodiment the polymer complexes of the present invention have been targeted to macrophages using biotin or analogues thereof as the targeting moiety. Once the drug has reached the inflammatory site, the complex is endocytosed by the target macrophage and the pendant drug may be released by the action of lysosomal enzymes, by cleavage of a disulfide linked drug by intracellular glutathione, or by the acid environment within intracellular compartments such as endosomes and lysosomes, or other means.

In a further embodiment, the polymer complexes of the present invention enhance the absorption of drugs from the intestine through the carrier-mediated biotin transport mechanism in the intestine.

This invention is not limited to any one specific hypothesis of the mechanism of action of the compositions described herein.

The polymer, P, of the present invention can be any pharmaceutically acceptable polymer. The polymer is able to be attached to at least one TM and to at least one, but preferably a multiplicity of active substance molecules.

Suitable polymers for substitution with biotin and modification according to the invention, include but are not limited to poly[N-(2-hydroxypropyl)-methacrylamide], dextran, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine) and branched chain polypeptides formed by the dual modification of the α and ϵ-amino groups of lysine during the peptide synthesis, as well as dendrimers and PEG-dendrimers, dextran, dextrin, glycosaminoglycans, carboxymethylcellulose, polylactic acid, polyglutamic acid, poly[lactide-co-glycolide], polyhydroxyethymethacrylate (poly-HEMA), and other such biodegradable, or non-biodegradable polymers. Such polymers may have multiple amino-termini, to which can be conjugated a plurality of the pharmaceutical or drug to be delivered. The polymers can also be formed with multiple cystines, to provide free thiols, or multiple glutamates or aspartates, to provide free carboxyls for conjugation using suitable carbodiimides. Similarly the polymer can contain multiple histidines or tyrosines

for conjugation. The polymer may have multiple hydroxyl groups suitable for modification, or alternatively may contain vicinal hydroxyl groups suitable for oxidation with reagents such as periodic acid.

In a further embodiment, the polymer can be degradable or biodegradable. Potentially biodegradable polymers include dextran and its derivatives, as well as dextrin, amino acid polymers such as poly lysine, poly-glutamic acid, alginate, heparin sulphate, and other sulphated polysaccharides, gelatin, glycosaminoglycans, preferred degradable polymers include poly(acetals), poly(anhydrides), and polymers of alpha-hydroxy acids such as poly[lactide-co-glycolide], It is within the scope of this invention for the polymer to be a block copolymer containing regions which are degradable and/or biodegradable, and regions which are stable

Non-biodegradable polymers may also be employed in the present invention and include, for example, poly[N-(2-hydroxypropyl)-methacrylamide]

In a preferred embodiment, the pharmaceutical is attached to the polymer through a biodegradable spacer such as those containing ester linkages, or amino acid sequences cleavable within lysosomal vacuoles, for example. Gly-Phe-Leu-Gly (Rihova, B. and J. Kopecek. 1985 Biological properties of targetable poly[N-(2-hydroxypropyl)-methacrylamide]-antibody complexes. J. Control Rel., 2 :289-310]. Other amino acid spacers cleavable by intracellular proteases include Gly-Phe-Ala; Gly-Phe-Ala-Gly; Gly-Phe-Tyr-Ala; and Gly-Phe-Tyr-Ala-Ala [Rejmanova, P., Obereigner, B., and Kopecek, J. 1981 Makromol. Chem. 182 : 1899-1915].

For anti-cancer applications, the carrier, biotin, or an analogue of biotin are preferably adapted to bind to surface biotin receptors on tumor cells.

Biotin is most easily covalently attached to a ligand, or the polymer, via the carboxylic acid moiety.

In one embodiment of the invention the linkage joining the pharmaceutical, or the biotin to the polymer is a disulfide bond. In a further embodiment of the invention the linkage joining the pharmaceutical, or the biotin to the polymer is an ester linkage. In yet another embodiment of the invention the linkage joining the pharmaceutical or the biotin to the polymer is a γ-glutamyl-ε-lysine bond. In yet another embodiment of the invention the linkage joining the pharmaceutical or the biotin to the polymer is a diazo-linkage. In yet a further example the bond linking the drug to the polymer is an acid labile linker, such as that formed with aconitic acid or via a hydrazone linkage.

The spacer compounds Q and Q' are optional. When they are absent the TM biotin, and/or the active substance A are linked to polymer P by a direct covalent bond. They are introduced either to improve the biotin receptor affinity of the biotin complex or to overcome problems in the coupling of the carrier, biotin, and/or the active substance A arising from unfavourable steric interactions between the biotin and A with the polymer p, or to increase the bioactivity of A in the complex. The spacer compounds may also act as linking agents, being bi-functional compounds with selected functional groups on each end to react with suitable functional groups located on the polymer, and also on the biotin carrier molecule and/or on the pharmaceutically active substances.

Suitable extended spacers for the conjugation of the pharmaceutical or biotin to the polymer matrix include : disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS), *N*-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), succinimidyl 4-(p-maleimidophyl)butyrate (SMPB).

Suitable cross-linking agents for use in the preparation of thiol-cleavable biodegradable linkers include N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

Nanoparticles

The complexes of the invention can also involve nanoparticles coated with biotin or an analogue thereof. Thus, amplification of drug/pharmaceutical delivery can occur by incorporation of the drug within biotin-coated nanoparticle. Accumulation of the biotin-nanoparticle-drug complexes in the kidneys is minimised due to the large size of the nanoparticle. Preferred nanoparticles comprise a nanosphere or nanocapsule, to which TM is coupled or attached, the nanosphere or nanocapsule enclosing an active agent, and wherein said targeting molecule (hereinafter termed TM) is biotin, or an analogue thereof, possessing binding activity for the biotin receptor. In a further embodiment, the nanoparticle is coated with a pharmaceutically-active substance (alone, or formulated with suitable excipients), and further coated with biotin or a biotin analogue. In another embodiment, the nanoparticle is coated with a mixture of a pharmaceutically-active substance and biotin or a biotin analogue (either this mixture alone, or formulated with suitable excipients)

The invention also provides a process for the production of a complex of the invention, which process comprises one or more of the following steps :

(a) reacting nanospheres with a TM to form the complex;

(b) chemically modifying a carrier molecule to provide at least one functional group capable of forming a chemical linkage and reacting nanospheres and the modified TM to form the complex;

(c) reacting nanospheres with at least one cross-linking agent to prepare "activated" nanoparticles which are reacted with a TM to form the complex;

(d) reacting a TM with at least one cross-linking agent and reacting the nanospheres with the reacted TM to form the complex;

(e) reacting nanospheres and a TM with at least one cross-linking agent to form the complex;

(f) reacting nanospheres with at least one cross-linking agent, reacting a TM with at least one cross-linking agent and reacting the reacted nanospheres and the reacted TM to form the complex; or

(g) reacting a TM with at least one cross-linking agent to prepare an analogue which is reacted with a hydrophobic moiety to form a hydrophobic derivative of the carrier; and then incubating the hydrophobic derivative of the TM with a nanosphere in such a manner that the TM is bound to the nanosphere by hydrophobic attraction.

Two basic forms of nanoparticles are known in the art, nanocapsules (or microcapsules) and nanospheres (or nanospheres), for enclosing, holding or containing an active substance. The active may be released from the nanoparticle to the circulatory or lymphatic drainage system, and most preferably to the target tissue of the host. These nanoparticle complexes may be used for oral delivery of the drug to the circulatory or lymphatic drainage system in general. The nanoparticles of this invention are also useful for targeting pharmaceutically-active compounds to sites of disease.

The terms "nanosphere" or "nanoparticle" as used throughout the specification refer to a small sphere or pellet ranging in size from 1 nanometre to 100 micrometers in size.

The term "carrier" as used throughout the specification refers to biotin or an analogue of biotin.

In essence the nanoparticles can be formed by any number of methods, several of which are outlined below:-

(i) Solvent Evaporation

In which a compound which is soluble in one solvent is dispersed into another miscible solvent and the first solvent is evaporated off. Particles formed in this fashion have been used to administer (parenterally) a number of water insoluble compounds. An example of such a system would be the formation of polyalkylcyanoacrylate nanocapsules in which the anticancer agent, 5-fluorouracil is entrapped.

(ii) Desolvation

In this method a compound is contained in a liquid in which it is soluble (the solvent) and a second liquid (which is miscible with the first liquid, but in which the compound is not soluble) is added to the solvent. As more of the second liquid is added the compound becomes desolvated. During the process of desolvation the compound rich phase (the coacervate) contains an enriched amount of compound which is dispersed as microdroplets in the compound deficient phase. At this stage the coalesced material can be chemically crosslinked by a suitable crosslinking agent to form micro- or nano-particles. Nanoparticles of gelatin or BSA can be prepared in this way. Solutions of these proteins are dessolvated by the addition of sodium sulfate, or ammonium sulfate solutions. At the point of desolvation there is an increase in turbidity, at which time the nanoparticles can be formed by the addition of a suitable cross-linker such as glutaraldehyde or butanedione. Alternatively a biodegradable cross-linker could be employed, such as a linker containing a disulfide bond, an azo-bond, or an esterase cleavable bond.

(iii) Complex coacervation

In this procedure two polyelectrolytes having opposite charge are mixed in aqueous medium so that a spontaneous liquid/liquid phase separation occurs. The phenomenon is limited to polymers having a suitable ionic charge density and chain length. Typically these nanospheres are formed by the addition of a polyanion such as Gum Arabic, Alginate, or Polyphosphate, to a polycation such as Gelatin. Suitable particles are readily formed by the complexation of gelatin and carboxymethyl cellulose. The rate of release of pharmaceutical from such complexes can be controlled by the addition of a suitable cross-linker such as glutaraldehyde or butanedione. Alternatively a biodegradable cross-linker

could be employed, such as a linker containing a disulfide bond, an azo-bond, or an esterase cleavable bond.

(iv) Polymer/polymer incompatability

This procedure is based upon the observation that two chemically different polymers dissolved in a common solvent are usually incompatible. Thus the mixture will tend to form two phases. The insoluble phase can be used to coat core particles to form microcapsules. An example would be the precipitation of ethyl cellulose from cyclohexane by the addition of polyethylene.

(v) Interfacial polymerization

In this technique, two reactants, each dissolved in a mutually immiscible liquid, diffuse to the interface between the two liquids where they react to form a capsule wall. An example of such capsule formation would occur if a mixture of Sebacoyl chloride dissolved in an oil phase and emulsified into an aqueous phase containing ethylenediamine.

The nanoparticle compositions of this invention may be formed by other methods familiar to those skilled in the art.

In another embodiment of the invention there is provided a complex between biotin and a biodegradable nanosphere in which is trapped a toxin or cytotoxic agent or active substance.

Polymers suitable for the formation of nanospheres by solvent evaporation (in liquid drying) include, amongst others, Poly-lactic acid, Poly-(Lactide/co-glycolide), Poly-hydroxybutyrate, Poly-hydroxyvalerate, Poly-(hydroxybutyrate/valerate), Ethyl cellulose, Dextran, Dextrin, Polysaccharides, Polyalkylcyanoacrylate, Poly-methyl-methacrylate, poly(e-caprolactone) and various combinations and co-polymers of the above.

Polymers suitable for the formation of nanospheres by interfacial precipitation/polymerization include, amongst others, EUDRAGIT™; Poly(N^{α},N^{ε}-L-lysinediylterephthaloyl); polymers formed by the reaction of Lysine hydrochloride and p-phthaloyl dichloride; by the reaction of acryloylated maltodextrin or acryloylated hydroxyethyl starch with ammonium peroxodisulfate and N,N,N',N'-tetramethylethylenediamine. Nanospheres can also be formed by the polymerization of various diamines such as ethylene diamine, phenylenediamine, toluene diamine, hexamethylene diamine, or diols such as ethylene diol, bisphenol, resorcinol, catechol, pentanediol, hexanediol, dodecanediol, 1,4 butanediol, with diacid chlorides such as sebacoylchloride and adipoyl chloride, or diisocynates such as hexamethylene diisocyanate using the methods fully described in EPA 85870002.4.

Polymers suitable for the formation of nanospheres by polymer phase separation include co-poly(vinyl chloride:vinyl alcohol:vinyl acetate), cellulosic polymers, polyvinyl acetate, polyvinyl alcohol, polyvinylchloride, natural and synthetic rubbers, polyacrylates, polystyrene and the like. Methods to synthesize such nanospheres are fully described in USP 4,166,800.

Polymers suitable for the formation of nanospheres by complex coacervation include, amongst others, mixtures of polyanions, such as gum arabic, alginate, carboxymethyl cellulose, carboxymethyl starch, polystyrene sulfonic acid, polyvinyl sulfonic acid, poly-D-glucuronic acid, Poly-pyruvic acid, carrageenan, heparin sulphate, polyphosphate with polycations, such as polylysine, gelatin.

Polymers suitable for the formation of nanospheres by Polymer/Polymer incompatability include, amongst others, ethyl cellulose, Ethylene vinyl acetate polymer, Poly(lactide), or Poly(vinylidene chloride) mixed with polymers such as Polyethylene, Silicone, Polyisobutylene or Polybutadiene.

Other materials suitable for formation of nanospheres include, Starch, Cross-linked Albumen, Polyacrylamide, Cross-linked gelatin and others obvious to those skilled in the art of nanosphere preparation.

The cross-linking agent may contain a disulfide bond or be cleavable by acid, base or periodate. Examples of suitable cross-linking agents include : N-(4-azidophenylthio)phthalimide; 4,4'-dithiobisphenylazide; dithiobis(succinimidylpropionate); dimethyl-3,3'-dithiobispropionimidate.2HCl; 3,3'-dithiobis-(sulfosuccinimidylpropionate); ethyl-4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimidate.HCl; N-succinimidyl-(4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate; sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate; N-succinimidyl-3-(2-pyridylthio)propionate; sulfosuccinimidyl-(4-azidophenyldithio)-propionate; 2-iminothiolane; disuccinimidyl tartrate; and bis-[2-(succinimidyloxycarbonyloxy)-ethyl]-sulfone.

Suitable linking of the carrier to the nanospheres may be achieved by reaction of the carrier with a carbodiimide and *N*-hydroxysuccinimide (NHS), and then reacting the NHS derivative with a suitable functional group on the nanosphere.

Biotin-targeting agents according to the invention are biotin molecules or analogues thereof that are able to be bound by or at least show binding to the biotin receptor. These biotin-targeting agents include, but are not limited to biotin, desthiobiotin, thioctic acid, diamino-biotin, biotin methyl ester, phosphonoacetyl-1'N-biotin, pantothenic acid, DL-pantoyl-taurine, D-pantethine, pantothenyl alcohol, iminobiotin, biocytin hydrazide, biotin hydrazide, biocytin, 5-(biotinamido)pentylamine, sulfo-NHS(n-hydroxysuccinimidyl)-biotin, sulfo-HNS-hexanyl-biotin (sulfo-NHS-LD-biotin), NHS-biotin, pentafluorophenyl-biotin, pentafluorophenyl-polyethylenoxide-biotin, NHS-biotin trifluoroacetamide, NHS-iminobiotin trifluoroacetamide, maleimido-polyethylenoxide biotin, maleimido-polyethylenoxide iminobiotin, and chloroacetyl-biotin, more preferably chloroacetyl-biotin.

The active substance to be delivered is preferably a hormone, drug, prodrug, cytotoxin, pharmaceutically active protein, immunogen, or DNA or RNA analogue.

Suitable toxins, according to the invention, include, but are not limited to, ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, ribosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin, maytansinoid.

Suitable cytotoxic agents, according to the invention, include, but are not limited to alkylating agents such as chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, 2-(hydroxymethyl)anthraquinone; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel. Other agents include DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

The compositions described herein, when used for the treatment of disease, may conceivably be used with or without the use of other pharmaceutical agents.

Compositions have been described herein possessing a single pharmaceutically-active ingredient, either attached or incorporated. It is within the scope of this invention for compositions to possess a plurality of pharmaceutically-active compounds, their derivatives and/or prodrugs, either attached or incorporated, such combinations of pharmaceutically-active compounds providing an additive or synergistic benefit in the treatment of disease.

Compositions have been described herein possessing a single biotin or biotin derivative either attached or incorporated. It is within the scope of this invention for compositions to

possess a plurality of biotin derivatives either attached or incorporated, such combinations of targeting groups providing an additive or synergistic benefit in the targeting of the compositions.

The terms "complex" and "macromolecular complex" are used herein in their broadest sense to include all forms of the biotin-mediated targeting compounds, compositions, complexes and associations between each one of a biotin targeting molecule or biotin analogue possessing binding activity to the biotin receptor, an active substance and a support, the support preferably being a polymer.

As used herein, the terms "treatment", "prophylaxis" or "prevention", "amelioration" and the like are to be considered in their broadest context. In particular, the term "treatment" does not necessarily imply that an animal is treated until total recovery. Accordingly, "treatment" includes amelioration of the symptoms or severity of a particular condition or preventing or otherwise reducing the risk of developing a particular condition.

The amount of the complex of the invention which is required in a therapeutic treatment according to the invention will depend upon a number of factors, which include the specific application, the nature of the particular compound used, the condition being treated, the mode of administration and the condition of the patient. The complexes may be administered in a manner and amount as is conventionally practised. The specific dosage utilised will depend upon the condition being treated, the state of the subject, the route of administration and other well known factors as indicated above. The length of dosing may range from a single dose given once every day or two, to twice or thrice daily doses given over the course of from a week to many months to many years as required, depending on the severity of the condition to be treated or alleviated. It will be further understood that for any particular subject, specific dosage regimens should be adjust over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

The production of pharmaceutical compositions for the treatment of the therapeutic indications herein described are typically prepared by admixture of the compounds of the invention (for convenience hereafter referred to as the "active compounds") with one or more pharmaceutically or veterinary acceptable carriers and/or excipients as are well known in the art.

Examples of pharmaceutically acceptable carriers, diluents and excipients for oral delivery include sodium bicarbonate solutions and similar diluents which neutralise stomach acid or have similar buffering capacity, glycols, oils or emulsions; and include medicaments in the form of gels, pastes and viscous colloidal dispersions. The medicament may be presented in capsule, tablet, slow release or elixir form or as a gel or paste. Furthermore the medicament may be presented as a food. Examples of pharmaceutically acceptable carriers, diluents and excipients for parenteral delivery include saline, glycols, oils or emulsions; and include medicaments in the form of gels, pastes and viscous colloidal dispersions.

In particular, the carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the subject. The carrier or excipient may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose, for example, a tablet, which may contain up to 100% by weight of the active compound, preferably from 0.5% to 59% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention, which may be prepared by any of the well known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory ingredients. The preferred concentration of active compound in the drug composition will depend on absorption, distribution, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art.

The formulations of the invention include those suitable for oral, rectal, optical, buccal (for example, sublingual), parenteral (for example, subcutaneous, intramuscular, intradermal, or intravenous) and transdermal administration, although the most suitable route in any

given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used.

Formulation suitable for oral administration may be presented in discrete units, such as capsules, sachets, lozenges, or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture such as to form a unit dosage. For example, a tablet may be prepared by compressing or moulding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound of the free-flowing, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Formulations suitable for buccal (sublingual) administration include lozenges comprising the active compound in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Compositions of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of the active compounds, which preparations are preferably isotonic with the blood of the intended recipient. These preparations are preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and

rendering the resulting solution sterile and isotonic with the blood. Injectable formulations according to the invention generally contain from 0.1% to 60% w/v of active compound and are administered at a rate of 0.1 ml/minute/kg.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations or compositions suitable for topical administration to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include Vaseline, lanoline, polyethylene glycols, alcohols, and combination of two or more thereof. The active compound is generally present at a concentration of from 0.1% to 5% w/w, more particularly from 0.5% to 2% w/w. Examples of such compositions include cosmetic skin creams.

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 M to 0.2 M concentration with respect to the said active compound. See for example Brown, L., *et al.* (1998).

Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, for example, Panchagnula R, *et al.*, 2000) and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable formulations comprise citrate or Bis/Tris buffer (pH 6) or ethanol/water and contain from 0.1 M to 0.2 M active ingredient.

Formulations suitable for inhalation may be delivered as a spray composition in the form of a solution, suspension or emulsion. The inhalation spray composition may further comprise a pharmaceutically acceptable propellant such as carbon dioxide or nitrous oxide.

The active compounds may be provided in the form of food stuffs, such as being added to, admixed into, coated, combined or otherwise added to a food stuff. The term food stuff is used in its widest possible sense and includes liquid formulations such as drinks including dairy products and other foods, such as health bars, desserts, etc. Food formulations containing compounds of the invention can be readily prepared according to standard practices.

Therapeutic methods, uses and compositions may be for administration to humans or animals, including mammals such as companion and domestic animals (such as dogs and cats) and livestock animals (such as cattle, sheep, pigs and goats), birds (such as chickens, turkeys, ducks), fish and other marine organisms, and the like.

The active compound or pharmaceutically acceptable derivatives, for example prodrugs or salts thereof, can also be co-administered with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or antiviral compounds. The active agent can comprise further drugs in combination or as a synergistic mixture.

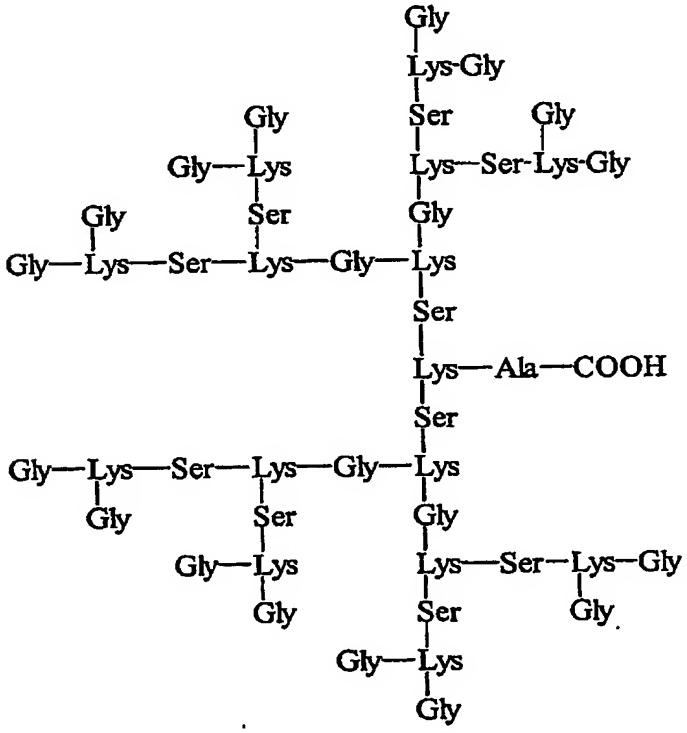
The co-administration may be simultaneous or sequential. Simultaneous administration may be effected by the compounds being in the same unit dose, or in individual and discrete unit doses administered at the same or similar time. Sequential administration may be in any order as required and typically will require an ongoing physiological effect of the first or initial active agent to be current when the second or later active agent is administered, especially where a cumulative or synergistic effect is desired.

The present invention is further described with reference to the following examples which are in no way limiting on the scope of the invention.

Example 1. Synthesis of Multi-Lysine polymer 1 (MLP1)

A multi-lysine polymer (MLP1) of the formula $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_5\text{-Ala-COOH}$, was synthesized on an Applied Biosystems peptide synthesiser. More precisely this represents $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_4[\text{Gly}_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]\text{-Ala-COOH}$

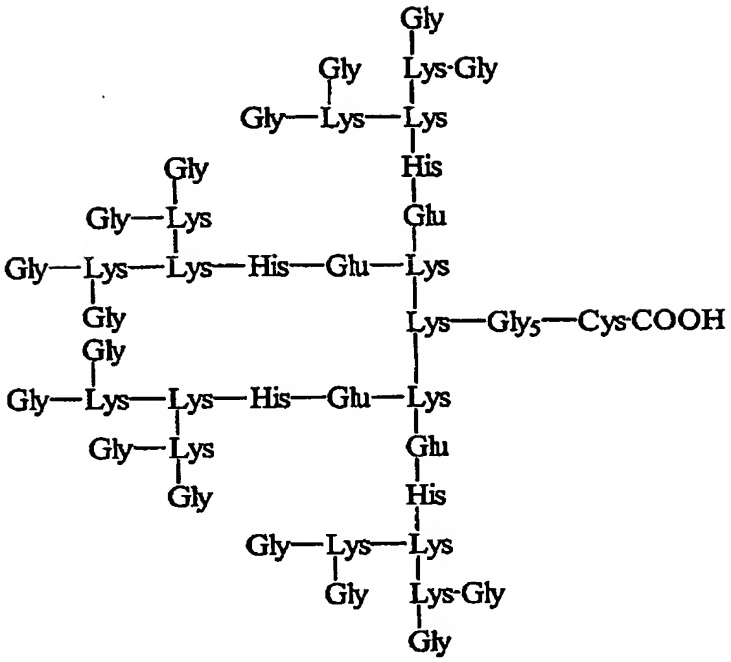
The formula $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_4[\text{Gly}_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]\text{-Ala-COOH}$ can be represented as follows :



which show the structure more precisely.

Example 2. Synthesis of Multi-Lysine polymer 2 (MLP2)

A multi-Lysine polymer (MLP2) of the general formula $[(NH_2\text{-Gly})_{16}\text{-Lys}_8\text{-Lys}_4\text{-His}_4\text{-Glu}_4\text{-Lys}_2\text{-Lys}]\text{-Gly}_5\text{-Cys-COOH}$ was synthesized on an Applied Biosystems peptide synthesiser. More precisely the structure can be represented as follows :



Example 3. Preparation of NHS-biotin.

Biotin (5g) was dissolved in 100 ml dry DMSO, plus 2.5 ml triethylamine. *N*-hydroxysuccinimide (2.6 gm) was added as a powder to the biotin and reacted overnight with 4.7 gm dicyclohexylcarbodiimide at room temperature. The dicyclohexylurea was removed by filtration. The DMSO was concentrated under reduced pressure and heating, and NHS-biotin precipitated with diethylether.

The product, was washed several times with anhydrous ether, dried under vacuum and stored as a white powder.

Example 4. Formation of MLP-toxin conjugates using biodegradable cross-linkers.

There are many toxins which could be used for formation of biotin-MLP-toxin conjugates including momordin, Pseudomonas exotoxin A, ricin and abrin. A general method for the formation of biotin-MLP-toxin conjugates is described below. Conjugates are prepared in which the covalent linker contains a biodegradable disulfide bond, which would be reduced *in vivo*, presumably by intracellular glutathione in the tumor cell, thereby releasing

the active substance after transport from the serum into the tumor cell. Briefly, MLP1 or MLP2 was reacted with N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). The dithiopyridyl-MLP (DTP-MLP) product was purified by RP-HPLC. A free thiol was introduced onto the toxin by a two step procedure in which the toxin was firstly reacted with SPDP, after which the thiopyridyl group was reduced with mercapto-ethanol. The product was purified by RP-HPLC. Alternatively free thiol was introduced into the toxin directly by reaction with iminothiolane. The thiolated product (SH-HN^+toxin) was purified by RP-HPLC. Formation of the disulfide linked MLP-toxin conjugates was achieved by reaction of the thiolated toxin derivative with DTP-MLP in 2.5% acetic acid for 24 hours. The conjugated material was purified by Sephadex G-25 chromatography, followed by RP-HPLC.

Example 5 Preparation of poly-drug-HPMA-biotin complex.

Two N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymers were synthesized as polymer backbones for the incorporation and derivatization with cytotoxic drugs and biotin. A non-biodegradable polymer backbone (HPMA-GG) was synthesized by the free radical copolymerization of HPMA with N-methacryloylglycylglycine p-nitrophenyl ester. A biodegradable polymer (HPMA-GFALG) was synthesized by the free radical copolymerization of HPMA with N-methacryloylglycylphenylalanylleucylglycine p-nitrophenol ester by the method of Rejmanova and co-workers [Rejmanova,P., Obereigner, B., and Kopecek, J. 1981 Makromol. Chem. 182 : 1899-1915]. In order to incorporate ricin A chain and biotin onto the polymers, they were reacted with a ten molar excess of a mixture of aminohexyl-biotin and Dithiopyridyldodecylsuberyl-hexylamine (1:10 mole:mole) overnight. Unreacted nitrophenyl esters were subjected to aminolysis by the addition of 1-amino-2-propanol. The modified polymers were purified by chromatography on Sepharose 6B. A solution of the dithiopyridyldodecylsuberylhexyl modified biotin-substituted polymers was dissolved in 2.5% acetic acid and reacted with ricin A chain. The

reaction mixture was left for 144 hours at 4°C, after which the ricin-biotin-substituted polymers were purified by chromatography on Sepharose 6B.

Example 6 Preparation of poly-daunomycin-HPMA-biotin complex.

An N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer was synthesized as a polymer backbone for the incorporation and derivatization with both the cytotoxic drug, daunomycin and biotin. A biodegradable polymer (HPMA-GFLG) was synthesized by the free radical copolymerization of HPMA with N-methacryloylglycylphenylleucinylglycine p-nitrophenol ester by the method of Rejmanova and co-workers [Rejmanova,P., Obereigner, B., and Kopecek, J. 1981 Makromol. Chem. 182 : 1899-1915]. In order to incorporate daunomycin and biotin onto the polymers, they were reacted with a ten molar excess of a mixture of aminohexyl-biotin and daunomycin (1:10 mole:mole) overnight. Unreacted nitrophenyl esters were subjected to aminolysis by the addition of 1-amino-2-propanol. The modified polymers were purified by chromatography on Sepharose 6B.

Example 7 Preparation of ^{125}I Labelled Polymers

Bolton-Hunter reagent was dissolved at 1 mg/ml in DMSO. The amino-derivatized polymer was dissolved at 5 mg/ml in DMSO or DW containing 25 µl/ml DIEA. A 3 µl aliquot of Bolton-Hunter was added to 20 µl of the polymer solution. The reaction was allowed to proceed for 3 hours. Unreacted Bolton-Hunter was extracted with DCM (5 × 100 µl) after addition of 50 µl water. ^{125}I (1 µl) was added to the derivatized polymer, followed by the addition of 4 µl Chloramine-T dissolved @ 20 mg/ml in PBS. The reaction proceeded for 15 secs, at which time the radioactive polymer was purified on PD10 column which had been equilibrated with 2.5% AcOH.

Example 8 Alternative Method of Preparation of Hydroxypropylmethacrylamide (HPMA)

1-Amino-2-propanol (58 g) was dissolved in acetonitrile (225 ml). The solution was cooled to –10 °C using an ethanol/dry ice bath. Methacryloyl chloride (40 g) in acetonitrile (170 ml) was added dropwise with vigorous stirring from a pressure equalising dropping funnel. The mixture was then allowed to warm slowly to room temperature overnight. The hydrochloride salt of 1-amino-2-propanol was removed by filtration through Celite filter aid. The solvent was removed at reduced pressure with a bath temperature of 50 °C. The product was isolated by dissolving in methanol and precipitation using acetone. The product was then dissolved in DW and dialysed extensively against DW.

Example 9 Preparation of Amino-HPMA

HPMA (4.0 g) was dissolved in DMSO (100 ml). A 1.5 ml aliquot of DIEA was added followed by 1.26 gm of solid CDI (1,1'-carbonyldiimidazole). The HPMA was activated for 45 min, whereupon an excess of 1,6-diaminohexane (4.0 g) was added. The reaction proceeded for 2 h, at which time the product was dialysed to remove unreacted amines. The final product was lyophilized.

Example 10 Preparation of Lysyl-HPMA

HPMA polymer (100K<MW<300K, 2.8 g) was dissolved in DMF (40 mL). DIEA (560 μL) was added, followed by Disuccinimidyl carbonate (1512 mg) and the mixture stirred at room temperature under N_2 overnight. Lysine was dissolved at 100 mg/ml in 10% sodium carbonate. 1 gm lysine was added to the derivatized-HPMA and allowed to react overnight. The product was purified by dialysis to remove free DSC and lysine.

Example 11 Preparation of Methotrexate-HPMA polymers targeted with biotin

HPMA polymer (100K<MW<300K, 2.8 g) was dissolved in DMF (40 mL). DIEA (560 μL) was added, followed by Disuccinimidyl carbonate (1512 mg) and the mixture stirred at

room temperature under N_2 overnight. Methotrexate-Gly-Phe-Leu-Gly-Lysine (630 mg) was added and the mixture stirred for 30 min.

Biotin-Lys (MW 372, 80 mg dissolved in 1% $NaHCO_3$ solution) was added and the mixture was reacted overnight. The Polymer-product was precipitated by the addition of ethyl acetate and the pellet collected by centrifugation at 5000 rpm. The pellet was washed twice with acetone, and the resultant product was dissolved in DW and dialysed extensively against ammonium hydrogen carbonate solution. The product was lyophilysed.

Example 12 Preparation of Methotrexate-Dextrin polymers targeted with biotin

Dextrin polymer (100K<MW<300K, 2.8 g) was dissolved in DMF (40 mL). DIEA (560 µL) was added, followed by Disuccinimidyl carbonate (1512 mg) and the mixture stirred at room temperature under N_2 overnight. Methotrexate-Gly-Phe-Leu-Gly-Lysine (630 mg) was added and the mixture stirred for 30 min.

Biotin-Lys (MW 372, 80 mg dissolved in 1% $NaHCO_3$ solution) was added and the mixture was reacted overnight. The Polymer-product was precipitated by the addition of ethyl acetate and the pellet collected by centrifugation at 5000 rpm. The pellet was washed twice with acetone, and the resultant product was dissolved in DW and dialysed extensively against ammonium hydrogen carbonate solution. The product was lyophilysed.

Example 13 Preparation of Aminohexyl-carboxymethyl cellulose (CMC)

CMC (low viscosity) was dissolved at 25 mg/ml in DW (2 gm/40 ml). NHS (150 mg dissolved @ 100 mg/ml in acetone) was added followed by 300 mg dry EDAC. The CMC was reacted for 15 minutes, whereupon 5 ml 1 M diaminohexane pH 9.5 was added and allowed to react O/WE. The product was dialysed exhaustively against DW. The product was then filter sterilized.

Example 14 Biotin Derivatisation of Polymers

Biotin (90 mg) was dissolved in DMSO (5.0 ml). DIEA (75 µL) was added, followed by TSTU ((O-(N-Succinimidyl)-N,N,N',N'-bis(tetramethylene)uronium hexafluorophosphate) (180 mg). The biotin was activated for 10 min, then 1.0 g Polymer (amino-HPMA, or amino-hexyl-CMC) dissolved in DMSO (50 ml) was added to the activated biotin solution and reacted overnight. The product was dialysed extensively to ensure removal of unreacted acid. The product was lyophilized.

Example 15 Preparation of methotrexate-GFLG-HPMA-Biotin

Methotrexate-GFLG-OH (FW 828, 36 mg, 3 x biotin) was dissolved in DMSO (5 ml). DIEA (20 µL) was added, followed by TSTU (35 mg). The methotrexate was activated for10 min. The polymer (100 mg) (Aminohexyl-HPMA or biotin-hexyl-HPMA) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilysed.

Example 16 Preparation of methotrexate-GFLG-CMC-Biotin

Methotrexate-GFLG-OH (FW 828, 36 mg, 3 x biotin) was dissolved in DMSO (5 ml). DIEA (20 µL) was added, followed by TSTU (35 mg). The methotrexate was activated for10 min. The polymer (100 mg) (Aminohexyl-CMC or biotin-hexyl-CMC) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilysed.

Example 15 Preparation of Clorambucil-GFLG-HPMA-Biotin

Chlorambucil-GFLG-OH (FW 678, 29 mg, 3 x biotin) was dissolved in DMSO (5 ml). DIEA (20 µL) was added, followed by TSTU (35 mg). The chlorambucil was activated for10 min. The polymer (100 mg) (Aminohexyl-HPMA or biotin-hexyl-HPMA) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilysed.

Example 16 Preparation of Chlorambucil-GFLG-CMC-Biotin

Chlorambucil-GFLG-OH (FW 678, 29 mg, 3 x biotin) was dissolved in DMSO (5 ml). DIEA (20 μL) was added, followed by TSTU (35 mg). The chlorambucil was activated for10 min. The polymer (100 mg) (Aminohexyl-CMC or biotin-hexyl-CMC) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilysed.

Example 17 Preparation of HPMA-hexylaminosuccinate

Aminohexyl-HPMA (300 mg) was dissolved in DMSO (5 ml) and succinic anhydride (100 mg) and DIEA (100 μL) added. The polymer was reacted overnight then dialysed extensively against DW and lyophilysed.

Example 18 Preparation of Daunomycin-GLFG-HPMA-biotin

HPMA-hexylaminosuccinic acid (35 mg) was dissolved in DMSO (2.0 ml). TSTU (18 mg) was added and activated for 10 min. H_2N-GFLG-Daunomycin (FW 938, 3 x biotin, 4.4 mg) was added and allowed to react for 5 min. For targeted polymers 6-aminohexyl-biotin (3 mg, designed to give 20% loading) was added and reacted for 1 h. The product was dialysed to remove unconjugated reagents. The final product was concentrated using an AMICON positive pressure stirred cell with 10K membrane.

Example 19 Preparation of MTX-GFLG-MLP-biotin

MTX-GFLG-OH (FW 828, 25 mg) was dissolved in DMSO (2 ml). TEA (5 μl) was added, followed by TSTU (15 mg, 1.2 equiv.). The reaction was allowed to proceed for10 min, afterwhich 13 mg MLP Polymer dissolved in DMSO (0.5 ml) was added and reacted for 60 min. For preparation of targeted polymers biotin (8 mg) dissolved in DMSO (0.8 ml) was activated with TSTU (8.5 mg) for 10 min and then the activated targeting agent was added to MTX-GFLG-MLP mixture. The reaction proceeded for 60 min. 0.1 M Tris pH 7.5 (5 ml) was added and stirred 1 h. The product was dialysed extensively and lyophilysed.

Example 20 Demonstration of biotin-mediated targeting of polymers.

In order to examine the potential utility of biotin as a targeting agent for polymer-drug conjugates, Lysyl-HPMA was substituted with rhodamine using rhodamine-isothiocyanate using standard methods. An aliquot of the Rho-HPMA was then further reacted with biotin, to produce a biotin-substituted-Rhodamine-HPMA. Control polymers were prepared without biotin. For tumour accumulation studies, various strains of mice bearing a variety of tumours were injected intraperitoneally with 5 mg/kg Rhodamine conjugated to the HPMA polymers. Six hours after injection, the mice were sacrificed, their tumours removed and cryo-embedded before cryostatic sectioning. The level of accumulation of the Rhodamine-HPMA was determined by fluorescent microscopy using a Zeiss microscope equiped with Axioplan software. Representative sections are shown in Figures 1 to 4.

The data depicted in Figures 1 and 2 show that the level of polymer uptake by P815 tumour cells can be enhanced by biotin derivatization of the rhodamine labelled polymers (red staining). Blue staining is BisBenzamide staining of cell nuclei.

The data depicted in Figures 3 and 4 show increased uptake of Biotin-HPMA by Colo-26 tumour cells.

Example 21 Increased Localization of targeted HPMA in L1210FR tumour cells in DBA/2 mice with Biotin.

Preliminary experiments were performed in L1210FR mice to see Rhodamine-labelled polymers would localize to ascites cells in L1210 FR tumours injected IP.

Methods

Lysyl-HPMA was derivatized with Fluorescein (using FITC) or rhodamine (using TRITC) using standard methods. Derivatization was aimed at 5 % substitution, however, with FITC

this was too substituted and resulted in an insoluble polymer, therefore substitution was backed off to 2.5%. For the production of targeted polymers the Glycyl-5'O-VB_{12}, and folate were activated with TSTU and used to substitute the remaining amino groups on the fluorescent polymers. Polymers were also biotinylated with NHS-biotin. Free reagents were removed by dialysis.

Mice were injected IP with 100 ug polymer and left for 5 hours, at which time the mice euthanased by cervical dislocation. The peritoneal cavity was then flushed with 5 ml of 3.8% trisodium citrate, and ascites fluid, containing cells, was then aspirated from the peritoneal cavity. The fluid was kept at 4°C ON before processing. The quantity of cells in the peritoneal wash out was determined by centrifuging the fluid and measuring the volume of the pellet. A fixed quantity of cells were then diluted out two-fold in an ELISA plate for measurement of fluorescence and determination of the level of uptake of fluorescent polymer.

Cells were also placed on slides for microscopic examination of internalized fluorescence. Fluorescence determination was performed on a Zeiss Axioplan fluorescent microscope, and photographed with

Results

Examination of the amount of fluorescent polymer taken up by isolated ascites cells taken from mice at the time of sacrifice showed greatly increased uptake of all targeted polymers. Greatest uptake was seen with the biotinylated polymers, followed by folate and vitamin B12 as targeting agents (see Figures 5 and 6).

Example 22 Preparation of nanospheres

Nanospheres can be formed by a number of techniques common to those knowledgeable in the art, including :- Solvent evaporation, Complex coacervation, Polymer/polymer incompatibility, Gelation, Interfacial polymerization and Thermal denaturation.

An effective amount of the complex is formulated with a pharmaceutically acceptable carrier, diluent or excipient to provide a medicament for administration to a patient requiring treatment of the conditions outlined in the body of the specification. The formulation is prepared using standard pharmaceutical techniques.

It is recognized that a number of factors will affect the determination of an appropriate dosage for a particular host. Such factors include the age, weight, sex, general health and concurrent disease states of the host. The determination of the appropriate dose level for the particular host is performed by standard pharmaceutical techniques.

Example 23 Preparation of nanospheres by Coacervation.

Almost any protein can be used as the matrix for entrapping drug via the desolvation technique, however preferred proteins according to the invention include bovine serum albumen (BSA), Ovalbumen (OA) and collagen,

BSA nanospheres formed by desolvation.
Nanospheres were prepared by coacervation of BSA following desolvation, according to the method of Oppenheim (Oppenheim, 1984, Oppenheim et al 1984, 1982), Briefly a 40% ammonium sulphate solution was added dropwise to a solution of 1% BSA containing 0.5% Tween 20 and the turbidity monitored by Klett readings, until the turbidity rose rapidly. At this point (determined by experimentation) the solution was placed in an ultra-turrax and 600 ul of glutaraldehyde added to cross-link the nanoparticles. Cross-linking was stopped by the addition of a solution of 12% sodium metabisulfite.

Particles were then washed extensively with distilled water prior to coupling to the NHS-derivative of biotin

Example 24 Incorporation of 5-fluorouracil

For incorporation of the antimitotic, 5-fluorouracil, 5-fluorouracil was dissolved at 10 g/100 ml of the BSA/Tween solution. Desolvation and cross-linking was carried out as described in Example 23.

Example 25 Coupling of biotin to nanospheres

Proteinaceous nanospheres (prepared by Method 23) were surface coated with biotin by reaction of biotin with EDAC and NHS followed by addition to the preformed nanospheres.

Example 26 Preparation of biotin-lipid complexes for hydrophobic insertion into nanospheres

In order to link biotin to the surface of nanospheres which have no readily available chemical groups suitable for chemical conjugation, it is possible to prepare a complex of biotin to an hydrophobic moiety which can insert, non-covalently, into the surface of the nanospheres. Such a molecule is easily added at the time of formation of the nanospheres. The strength of the hydrophobic association is such that there is only a very slow dissociation of the biotin from the nanospheres under physiological conditions.

a) Preparation of biotin-phosphatidyl ethanolamine (biotin-PEA)
Phosphatidylethanolamine (100mg) was dissolved in 2 ml chloroform/methanol (50:50, v/v). Biotin (100 mg) was added to the mixture. The biotin was then cross-linked to the PEA by the addition of 200 mg of the carbodiimide, 1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide (EDC or EDAC). The reaction was allowed to proceed for 90 minutes prior to the addition of the biotin-PEA to nanospheres.

b) Preparation of other complexes between biotin and an hydrophobic moiety.
Covalent complexes can be made between analogues of biotin and almost any aliphatic or aromatic chains or amphipathic containing a water soluble head group suitable for conjugation and a lipid soluble tail suitable for hydrophobic association within an

hydrophobic environment. Thus, any lipid (saturated, unsaturated or polyunsaturated) which has a carboxylic acid head group, such as oleic acid, octanoic acid, linoleic acid or glycerophophoric acids may be directly conjugated to an amino-biotin derivative using a suitable carbodiimide (EDAC or DCC, for example). Similarly any amphiphathic molecule possessing an amino-group (amino-hexane, amino-decane, amino-dodecane, phosphatidyl-ethanolamine, may be conjugated directly to carboxy-biotin using carbodiimides.

Example 27 Preparation of biotin-Nanospheres by solvent evaporation.

a) Preparation of biotin-PEA-[Polymethylmethacrylate] nanospheres

Polymethylmethacrylate (PMM, Polysciences)(MW 12,000; 500mg) was dissolved in 2 ml of dichloromethane (DCM). The PMM in DCM was then added dropwise to 20 ml of 0.25% Polyvinylalcohol (PVA) while homogenizing at 13,500 rpm with a Janke & Kunkel Ultraturrax. After 1 minute, 200 ul of biotin-PEA was added and stirred gently overnight. The nanospheres were then harvested by centrifugation, washed three times with water and lyophilized.

b) Preparation of biotin-[PEA-Poly-lactic acid] nanospheres.

Poly-lactic acid (PLA, Polysciences)(MW 50,000; 500mg) was dissolved in 3 ml of DCM and then homogenized into 20 1% PVA at 13,500 rpm on Ultraturrax T25 with an S25F probe for 5 minutes. Biotin-PEA (400 ul) was added while the solution was stirred gently. Nanospheres were harvested as described above.

c) Preparation of biotin-PEA-[Poly-Hydroxy-butyrate/valerate] nanospheres

Poly-Hydroxy-butyrate/valerate (9% valerate) (ICI; 500 mg) was dissolved in 5 ml of DCM and homogenized into 20 ml 1% PVA at 13,500 rpm on Ultraturrax T25 with an S25F probe for 5 minutes. Biotin-PEA (400ul) was added and the spheres processed as described in 8b.

Example 28 Covalent conjugation of biotin to nanospheres with surface carboxyl groups.

A general method for the conjugation of biotin to the surface of nanospheres made from polymers with free carboxyl groups is outlined below. The specific example utilizes commercially available carboxyl-modified nanospheres.

Polysciences Fluoresbrite™ carboxylate Nanospheres (2.5% Solids Latex) were obtained from Polysciences in sizes of 0.045um, 0.49um, 2.2um and 9.97um. One ml of each of the preparations was washed extensively with DW and resuspended in 200 ul of distilled water. To each preparation was added 1.5 mg aminohexyl biotin then 5 mg of EDAC. Each preparation was allowed to react overnight, after which unreacted material was removed by repeated washing with DW or by dialysis against DW.

Example 29 Surface derivatisation of nanospheres

Many polymers used in the preparation of nanospheres by solvent evaporation do not contain functional groups for direct conjugation to biotin or its functionalised analogues, however it is possible to modify the surface of the preformed nanospheres to introduce functional groups suitable for conjugation to biotin.

a) Surface derivatisation of Polylactic acid (PLA) nanospheres

Preformed PLA nanospheres (10 mg) were gently suspended in distilled water (DW; 350 ul) by rotation on a rotary shaker for 2 hours. Hydrazine hydrate (10 ul) was added and the suspension was shaken overnight at room temperature. The spheres were spun down and repeatedly washed with water by re-suspension and centrifugation. The washing procedure was repeated until the supernatant failed to give a positive hydrazine test (purple colour upon reaction with a solution of TNBS; 1 mg /ml). The spheres were washed a further two times and the wet pellet used directly for conjugation to biotin.

b) Conjugation of biotin to hydrazine modified PLA nanospheres

A sample of the hydrazine modified PLA nanospheres (3ul wet pellet) was suspended in DW (250ul). Aqueous solutions of biotin (10 mg/ml, 400ul) and EDAC (100 mg/ml, 100

ul) were added and the reaction mixture shaken overnight at room temperature. The suspension was spun down and the supernatant removed. The pellet was washed repeatedly with DW (6 washes). The residual pellet was then vacuum dried.

Two control reactions were performed concurrently with the above conjugation. In the first a 3 mg sample of hydrazine-modified PLA nanospheres was treated with the biotin as described above but DW was used in place of the EDAC solution. In the second control a 2 mg sample of unmodified PLA nanospheres was treated with both biotin and EDAC as described above. For both controls the pellet remaining after repeated washing was a clear white colour with no evidence of any associated biotin.

Example 30 Preparation of Isobutyl-cyanoacrylate Nanocapsules, surface-coated with biotin

Nanocapsules suitable for biodistribution studies were prepared with ^{125}I-insulin as ant internal marker. Briefly, 10 mg insulin was dissolved at 10mg/ml in 0.1M HCl. An aliquot (1µl) of ^{125}I-insulin was added to the cold insulin, which was mixed with 100µl miglyol and vortexed. EtOH (10 ml) was added to the insulin/miglyol mix and mixed by vortexing. IBCA (100 µl, Sicomet) was added to the clear solution, which was immediately added to 60 ml 0.25% F-127. After 30 minutes the preparation was split into 2 equal halves. One half was left to stir overnight, whilst to the other half was added 27mg biotin-PEG-octadecanoic acid (80mg/ml in EtOH). The solution was left to stir overnight. Both solutions were then treated in a similar fashion. Large aggregates were removed by centrifugation at 10K for 20 minutes. Both particle preparations were concentrated and washed in a Amicon positive pressure filtration unit using a 300,000 MW cut off membrane. Particles were stabilized by surface cross-linking with di-succinimidyl-2-aminoethyl-2-amino-2-benzyl-ethanoate (DSAB). DSAB was converted to the NHS-ester as follows. DSAB (40 mg) was dissolved in an equal weight of DMF, to which was added NHS (24mg, 240µl DMF). DCC (Dicyclohexylcarbodiimide, 44mg, 440µl, made up fresh) was then added to the DSAB mixture and allowed to activate for 20' while stirring rapidly.

The DSAB-NHS-ester was added at 0.32mg per 2.1mg nanocapsules, and left to stir O/N. The particles were then dialysed before use in biodistribution studies.

Industrial Applications

The present invention provides a simple and novel technique for the specific targeting of pharmaceuticals to tumour cells using polymers and nanoparticles. The invention also provides a method for the amplification of the biotin uptake system. The present invention further provides a simple and novel technique for the specific protection of active substances during their transit down the intestine. Thus the present invention provides a simple and novel technique for the specific protection of active substances from enzymatic degradation as well as for amplification of the biotin uptake system thus enabling a wide range of active agents to be actively absorbed intact from the intestine. The invention also provides a complex suitable for administration for the treatment of tumour cells, which over-express receptors involved in biotin uptake. Additionally the invention provides complexes suitable for administration to a vertebrate host to treat inflammatory conditions.

The invention has been described herein, with reference to certain preferred embodiments, in order to enable the reader to practice the invention without undue experimentation. However, a person having ordinary skill in the art will readily recognise that many of the components and parameters may be varied or modified to a certain extent without departing from the scope of the invention. Furthermore, titles, headings, or the like are provided to enhance the reader's comprehension of this document, and should not be read as limiting the scope of the present invention.

The entire disclosures of all applications, patents and publications, cited herein, if any, are hereby incorporated by reference.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also

includes all of the steps, features, compositions and compounds referred to or indicated in this specification individually or collectively, and any and all combinations of any two or more of said steps or features.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in the field of endeavour.

Selected References

Oppenheim R.C. (1984) in "Polymeric Microparticles" (Guiot, P and Couvreur, P. Eds.) CRC Press, Boca Raton.

Oppenheim R.C., Gipps, E.M. Forbes, J.F. and Whitehead R.H. (1984) in "Nanospheres and Drug Therapy" (Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. Eds) Elsevier Science Publishers B.V.

Oppenheim, R.C., Stewart, N.F., Gordon, L. and Patel, H.M. (1982) Drug Devel. Indust. Pharm. 8: 531-546.

Allen, R.H. and Majerus, P.W. (1972) J.Biol. Chem. 247: 7702-7717.

Yamada, R.-H and H.P.C. Hogenkamp. (1972) J.Biol.Chem. 247 : 6266-6270.

Claims

1. A macromolecular complex comprising a support to which is coupled a targeting molecule in association with an active substance, wherein said targeting molecule is biotin or an analogue thereof possessing binding activity to the biotin receptor.

2. A complex of claim 1, wherein the support is a polymer.

3. A complex of claim 2 having the general formula

$$(B\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$$

wherein, B is biotin or a derivative thereof which will bind to a natural biotin receptor
n is a number from 1.0 to about 50;
P is a pharmaceutically acceptable polymer;
A is a pharmaceutically active substance;
m is a number greater than 1.0 to about 1000; and
Q and Q' are independently a covalent bond, or a spacer compound linking biotin, P and A by covalent bonds.

4. A complex according to claim 3, wherein at least one of Q and Q' is a spacer compound which contains a biodegradable portion.

5. A complex according to claim 4, wherein said biodegradable portion is selected from a disulfide bond, ester linkage, a γ-glutamyl-ϵ-lysine linkage and a diazo bond, and Gly-Phe-Leu-Gly.

6. A complex according to claim 3, wherein n is from 1.0 to about 1.5 and m is from 2 to about 200, more preferably from about 10 to 100.

7. A complex according to claim 3, wherein P is a biodegradable polymer.

8. A complex according to claim 7, wherein said biodegradable polymer is selected from a biodegradable carbohydrate polymer or a polymer of amino acids.

9. A complex according to claim 7, wherein said polymer is a non-biodegradable.

10. A complex according to claim 9, wherein said non-biodegradable polymer comprises biodegradable side chains for covalent linkage to an active substance.

11. A complex according to claim 3, wherein said polymer is selected from poly[N-(2-hydroxypropyl)-methacrylamide], dextran or dextran derivatives, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine), branched chain polypeptides, carboxymethyl cellulose, dendrimers and PEG-dendrimers.

12. A polymer according to claim 11, wherein said polymer is a branched chain polypeptide optionally modified to provide multiple functional groups for coupling of an active substance.

13. A complex according to claim 4, wherein said spacer compound Q or Q' has from 1 to 50 atoms in its backbone.

14. A complex according to claim 3, wherein said diradical spacer comprises optionally substituted alkylene C_{1-50} moiety optionally contained within the chain, double bonds, triple bonds, aryl groups and or hetero atoms.

15. A complex according to claim 14, wherein said spacer compound is derived from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-

ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

16. A complex according to claim 14, wherein said spacer compound is thiol cleavable.

17. A complex according to claim 16, wherein said thiol-cleavable spacer is derived from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

18. A complex according to claim 3, wherein the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_n$-Ala-COOH, where n= 1 to 85.

19. A complex according to claim 3, wherein the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-X}_o)_4\text{-Lys}_2\text{-Y}_2\text{-Lys}]_n\text{-Z}_m$-COOH, where n= 1 to 85; m= 1 to 10; o = 1 to 10; where X is any amino acid, where Y is any amino acid, and where Z is any amino acid.

20. A complex according to claim 3, wherein the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-Gly})_{16}\text{-Lys}_8\text{-Lys}_4\text{-His}_4\text{-Glu}_4\text{-Lys}_2\text{-Lys}]_n\text{-Gly}_m$-Cys-COOH, where n= 1 to 85; where m = 1 to 10.

21. A complex according to claim 3, wherein the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-X})_{16}\text{-Lys}_8\text{-Lys}_4\text{-Y}_4\text{-Z}_4\text{-Lys}_2\text{-Lys}]_n\text{-AA}_m$-Cys-COOH, where

n= 1 to 85; where m = 1 to 10; where X, Y, Z and AA represent any amino acid independent of each other.

22. A complex according to claim 3, wherein the pharmaceutically acceptable polymer is poly[N-(2-hydroxypropyl)-methacrylamide].

23. A process for the production of a complex having the general formula

$$(B\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$$

wherein B, Q, P, Q', A, n and m are as defined in claim 3, said process selected from any one or more of the following steps:

a) reacting A with P to form an intermediated complex, and thereafter reacting the intermediate complex with B;

b) reacting B with P to form an intermediate complex and thereafter reacting the intermediate complex with A;

c) the process of step a) or step b) wherein one or more of B, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; and

d) reacting one or two of B, P or A with Q and/or Q' prior to coupling with the other reactants.

24. A process according to claim 23, wherein Q and/or Q' comprises an optionally substituted alkylene $C_{1\text{-}50}$ moiety optionally within the chain, double bonds, triple bonds, aryl groups, and/or hetero atoms.

25. A process according to claim 23, wherein Q' is a cleavable cross-linking agent containing a disulfide bond.

26. A process according to claim 25, wherein the cross-linking agents are selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate)

(Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

27. A process according to claim 23, wherein said spacer is selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

28. A process according to claim 23, wherein said spacer is selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

29. A process according to claim 25, wherein the cross-linking agents are selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-

pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

30. A complex prepared by a process of claim 23.

31. A pharmaceutical composition which comprises a complex according to any one of claims 1 to 23 or claim 31 together with a pharmaceutically acceptable carrier or excipient.

32. A complex of claim 1, wherein the support is a nanoparticle and the active agent is enclosed in the nanoparticle and/or adhered to the surface of the nanoparticle.

33. A complex of claim 32, wherein the nanoparticle is prepared by solvent evaporation, complex coacervation, polymer/polymer incompatibility, gelation, interfacial polymerisation or thermal denaturation.

34. A complex of claim 32 wherein the nanoparticle is biodegradable.

35. A process for the production of a complex of claim 32, which process comprises one or more of the following steps:

a) reacting nanospheres with a targeting molecule to form the complex;
b) chemically modifying a targeting molecule to provide at least one functional group capable of forming a chemical linkage and reacting nanospheres and the modified targeting molecules to form the complex;
c) reacting nanospheres with at least one cross-linking agent to prepare "activated" nanoparticles which are reacted with a targeting molecule to form the complex;
d) reacting a targeting molecule with at least one cross-linking agent and reacting the nanospheres with the reacted targeting molecule to form the complex;
e) reacting nanospheres and a targeting molecule with at least one cross-linking agent to the complex;

f) reacting nanospheres with at least one cross-linking agent, reacting a targeting molecule with at least one cross-linking agent and reacting the reacted nanospheres and the reacted targeting molecule to form the complex; or

g) reacting a targeting molecule with at least one cross-linking agent to prepare an analogue which is reacted with a hydrophobic moiety to form a hydrophobic derivative of the targeting molecule, and then incubating the hydrophobic derivative of the targeting molecule with a nanosphere in such a manner that the nanosphere is coated hydrophobically with the targeting molecule.

36. A process of claim 35, wherein the cross-linking agent contains a disulfide bond or is cleavable by acid, base or periodate.

37. A process of claim 35, wherein the cross-linking agent is selected from the group consisting of N-(4-azidophenylthio)phthalimide, 4,4'-dithiobisphenylazide, dithiobis(succinimidylpropionate), dimethyl-3,3'-dithiobispropionimidate.2HCl, 3,3'-dithiobis-(sulfosuccinimidylpropionate), ethyl-4-azidophenyl)-1,3'dithiopropionate, sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimidate.HCl, N-succinimidyl-(4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate, N-succinimidyl-3-(2-pyridylthio)propionate, sulfosuccinimidyl-(4-azidophenyldithio)-propionate, 2-iminothiolane, disuccinimidyl tartrate and bis-[2-(succinimidyloxycarbonyloxy)-ethyl]-sulfone.

38. A process of claim 35, wherein the targeting molecule is cross-linked to the nanosphere or nanoparticle by reaction of the carrier with a carbodiimide and *N*-hydroxysuccinimide (NHS), and then reacting the NHS derivative with a suitable functional group on the nanosphere.

39. A process of claim 35, wherein the cross-linking agent contains a biodegradable bond

40. A process of claim 39, wherein the cross-linking agent is cleaved by an esterase, glutathione, or azo-reductase.

41. A complex prepared by a process of claim 35.

42. A pharmaceutical composition comprising a complex of claim 32 or claim 41 in association with a pharmaceutically acceptable carrier, excipient or diluent.

43. A complex according to claim 1 wherein said active substance A is a biologically active toxin or a part thereof.

44. A complex according to claim 43, wherein said toxin is selected from ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, ribosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin, maytansinoid.

45. A complex according to claim 43, wherein said alkylating agents are selected from chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, [2-(hydroxymethyl)anthraquinone]; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platimun, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel.

46. A complex according to claim 43, wherein said alkylating agents are selected from DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

47. A complex according to claim 1, wherein said biotin or analogue thereof is biotin, desthiobiotin, thioctic acid, diamino-biotin, biotin methyl ester, phosphonoacetyl-1'N-biotin, pantothenic acid, DL-pantoyl-taurine, D-pantethine, pantothenyl alcohol,

iminobiotin, biocytin hydrazide, biotin hydrazide, biocytin, 5-(biotinamido)pentylamine, sulfo-NHS(n-hydroxysuccinimidyl)-biotin, sulfo-HNS-hexanyl-biotin (sulfo-NHS-LD-biotin), NHS-biotin, pentafluorophenyl-biotin, pentafluorophenyl-polyethylenoxide-biotin, NHS-biotin trifluoroacetamide, NHS-iminobiotin trifluoroacetamide, maleimido-polyethylenoxide biotin, maleimido-polyethylenoxide iminobiotin or chloroacetyl-biotin.

48. A method for the treatment or prophylaxis of disease which comprises administering to a subject a therapeutically effective amount of a complex according to claim 1 or a composition according to claim 31 or 42.

49. A method of claim 48, wherein the disease is cancer.

50. A method of claim 48, wherein the disease is rheumatoid arthritis.

51. A method of claim 48, wherein the disease is Crohn's disease.

52. A method of claim 48, wherein the disease is inflammatory bowel disease.

53. A method of claim 48, wherein the disease is multiple sclerosis.

54. Use of a complex according to claim 1 in the manufacture of a medicament.

55. A macromolecular composition of claim 1 or claim 32 which is orally administered in a pharmaceutically-acceptable formulation, whereby the macromolecular composition of claim 1 or claim 32 is absorbed in the intestinal lumen

56. A macromolecular composition of claim 55 in which intestinal lumen absorption is enhanced by a process involving the recognition of biotin by a Na^+-dependent, carrier-mediated mechanism for biotin uptake in the small intestine.

57. A macromolecular composition of claim 55 in which the macromolecular composition is transported from the intestinal lumen to the bloodstream and/or the lymphatic system.

DATED this 21st day of November 2002

ACCESS PHARMACEUTICALS AUSTRALIA PTY LIMITED
By its Patent Attorneys
DAVIES COLLISON CAVE

Fig 1

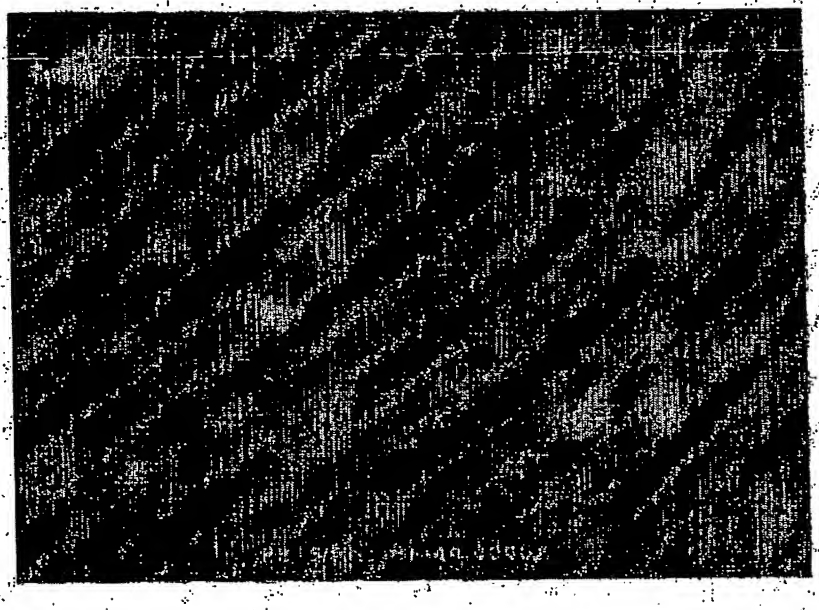


Fig 2

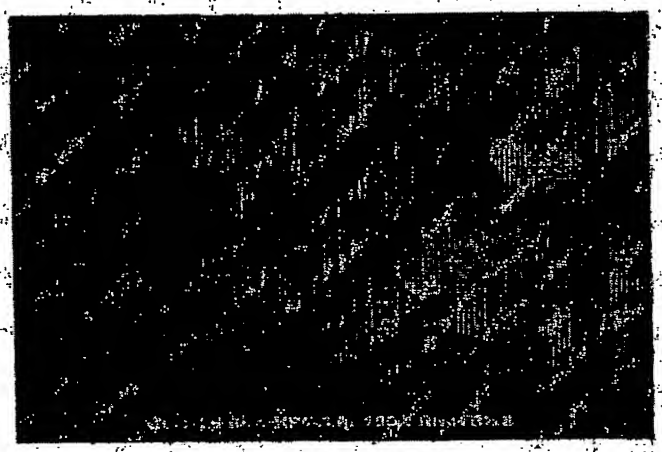


Fig 3

Fig 4

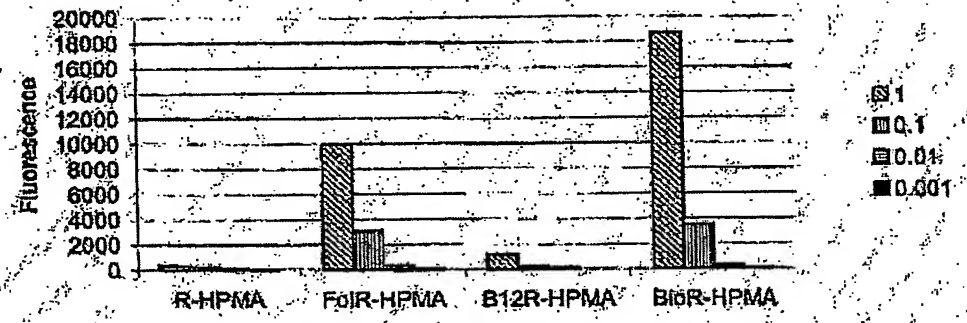

20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Fluorescence
R-HPMA
FolR-HPMA
B12R-HPMA
BioR-HPMA
1
0.1
0.01
0.001

Fig 5

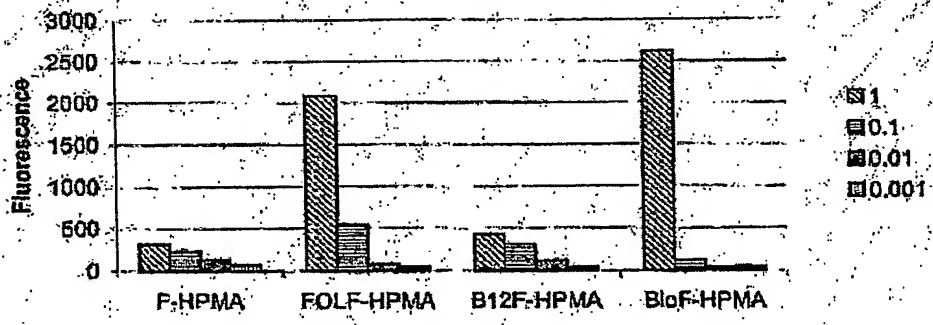

3000
2500
2000
1500
1000
500
0
Fluorescence
F-HPMA
FOLF-HPMA
B12F-HPMA
BioF-HPMA
1
0.1
0.01
0.001

Fig 6